



Technical Note 144

High Sensitivity Assay Standard Protocol

Introduction

The DeNovix dsDNA High Sensitivity Assay enables the accurate detection of purified double-stranded DNA (dsDNA) samples with a standard detection range from 100 pg to 250 ng total mass in 200 μ L volumes. This equates to sample concentrations of 10 pg/ μ L – 250 ng/ μ L when using between 1 – 20 μ L sample volumes in 200 μ L assay volumes. The assay is linear for sample concentrations as high as 250 ng/ μ L when adjusting volumes to 1 μ L of sample into 199 μ L of working reagent. Total mass should not exceed 250 ng for best results.

The lower detection limit can be extended down to 5 pg/ μ L sample by adding 20 μ L of the 5 pg/ μ L sample to 180 μ L of the working reagent.

[View Detailed Protocol](#)

Kit Contents

Kits are available in 1000, 250 and 50 (evaluation size) assays and include the components in Table 1. Safety data sheets are available at denovix.com/sds.

Component	1000	250	EVAL
DeNovix dsDNA High Sensitivity Dye (100x)	2 x 1 mL	0.5 mL	100 μ L
DeNovix dsDNA High Sensitivity Buffer	200 mL	50 mL	10 mL
25 ng/ μ L dsDNA Standard (calf thymus)	2 mL	1 mL	0.5 mL
0 ng/ μ L dsDNA Standard	2 mL	1 mL	0.5 mL

Best Practices

- Prepare the working solution fresh for each assay. Discard the solution after 24 hours.
- Use properly calibrated pipettes and DNase-free pipette tips.
- Treat all standards and samples identically in terms of incubation times and temperature.
- Avoid introducing air bubbles when mixing.
- Generate a new standard curve for each assay.
- Ensure that sample solution contaminant levels are compatible with the assay.
- Assay total mass must be considered when deciding how much sample to use. This assay is appropriate for 100 pg – 250 ng total mass per tube.
- Label only the tops of the tubes.

Sample Prep

1. Allow all solutions to equilibrate to room temperature before use. Vortex, then centrifuge vials briefly before opening to minimize reagent loss on the cap.
2. Prepare working solution by mixing the dye with the assay buffer in a 1:100 ratio, e.g. 100 μ L dye into 10 mL buffer. Scale volumes as needed to make enough volume to aliquot 190 μ L of the mixture for each standard and unknown. Discard after 24 hours.
3. For each standard or unknown sample, add 190 μ L of the working solution into a labeled tube. Adjust volume when adding more or less than 10 μ L of the unknown sample.
4. Use thin-walled, clear 0.5 mL PCR tubes for assay measurements (DeNovix cat #TUBE-PCR-0.5-500 or equivalent).
5. Add 10 μ L of the 0 ng/ μ L, 25 ng/ μ L standards or 1 – 20 μ L of unknown DNA samples to the respective tubes and mix well.
6. Incubate assay tubes at room temperature for 5 minutes.

Recommended Sample Volume

These recommendations ensure that sample concentrations are within the total mass detection limits of the assay. Total assay volume should remain 200 μ L. Adjust working solution volume accordingly.

Initial Sample Concentration	Recommended Sample Volume
10 pg/ μ L – 25 ng/ μ L	10 μ L
5 – 100 pg/ μ L	20 μ L

Initial Sample Concentration Recommended Sample Volume

25 – 125 ng/μL	2 μL
125 – 250 ng/μL	1 μL

Sample Measurement

1. Launch the Fluoro dsDNA app using a DeNovix Fluorometer.
2. Use the drop-down menu to select the correct LED source for the DeNovix dsDNA High Sensitivity Assay.
3. Select the preferred standard curve method (2 point standards supplied) and then choose Generate New Standard Curve.
4. Insert the 0 ng/μL dsDNA standard tube, lower the lid and tap the **Measure** button.
5. Insert the 25 ng/μL dsDNA standard tube, lower the lid and tap the **Measure** button.
6. After both standards are measured, tap the Samples button, insert a sample tube and tap **Measure**.

Reagent Storage

Component	Protect from Light	Temperature
DeNovix dsDNA High Sensitivity Dye (100x)	Yes	4°C - Room Temperature
DeNovix dsDNA High Sensitivity Buffer	Optional	4°C - Room Temperature
dsDNA Standards	Yes	4°C

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